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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/523,459	01/31/2005	Noel Martin Young	025786-000100US	7643

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EXAMINER	
PORTNER, VIRGINIA ALLEN	
ART UNIT	**PAPER NUMBER**
1645	
MAIL DATE	**DELIVERY MODE**
08/09/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/523,459	YOUNG ET AL.	
	Examiner	Art Unit	
	Ginny Portner	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) *6 and 10-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5 and 7-9* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *31/31/05 figure 3/12* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *sequence letter attachments* / Notice to Comply

DETAILED ACTION

Claims 1-32 are pending.

Election/Restrictions

1. Applicant's election with traverse of Group I in the reply filed on May 8, 2007 is acknowledged. The traversal is on the ground(s) that the reference used in formulating the lack of unity is in fact not prior art and all of the groups should be rejoined except group II, which is not the same invention as the others. This is found partially persuasive because all of the species of group I can be searched without undue burden on the examiner, but as seen below, prior art was found that are anticipatory of the first appearing invention which includes immunologically active fragments of the recited compound, as well as a glycoprotein of Campylobacter jejuni that is glycosylated with a hepatasaccharide (1999 reference) that anticipates the first appearing invention. The lack of unity requirement is still deemed proper and is therefore made FINAL. Claims 1-5 and 7-9 are under examination.

2. Claims 6 and 10-32 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on May 8, 2007.

3. The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of

the allowable product claim will be considered for rejoinder. All claims directed to a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**. Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Information Disclosure Statement

1. The information disclosure statement filed July 25, 2005 has been considered.

Sequence Letter/Sequence Requirements

2. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Full compliance with the sequence rules is required in response to this office action. Failure to fully comply with these requirements in the time period set forth in this office action will be held non-responsive.

3. Sequences shown on pages 15,16,18 and 20; as well as in figure 3/12 must have sequence identifiers inserted in the Specification and the Brief Description of the Drawings, respectively, (SEQ. ID. NO) to be in sequence compliance.

Claim Rejections - 35 USC § 101

4. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

5. Claims 1-5 are not isolated and purified compounds and therefore do not show the hand of man; the claimed invention is directed to non-statutory subject matter. While claim 4 is directed to a product defined by product-by-process language, the product compound is not isolated and purified, and therefore still reads on a product of nature. This rejection could be obviated by amending the claims to recite the phrase -----An isolated and purified compound-----or --------A composition comprising an isolated and purified compound---------.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

7. Claims 1,2,4-5 are rejected under 35 U.S.C. 102(a) as being anticipated by Linton et al (January 2002).

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

Linton et al disclose the instantly claimed invention directed to a compound that comprises N-acetylgalactoseamine (see title), the compound being from Campylobacter jejuni (see title), and is linked to an amino acid, either serine or threonine (see page 505, line 1) in a protein (PEB3 or CgpA, see page 505, col. 1, paragraph 3) of Campylobacter jejuni (see "glycoprotein", title). The N-acetylgalactoseamine compound is immunologically active as it induced antibodies and was immunoreactive (see page 502, col. 2, last paragraph, figure 6(a) page 503; page 504 "they are highly immunogenic proteins with antibodies raised against glycan

rather than amino acid epitopes (Szymanski et al, 1999"). Linton et al anticipates the instantly claimed invention as now claimed.

8. Claims 1-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Szymanski et al (1999) in light of evidence provided by Guerry et al (US PG-Pub 2007/0065461).

Szymanski et al disclose the instantly claimed invention directed to compounds that comprise:

9. **Instant claim 1**: a heptasaccharide in light of evidence provided by Guerry et al (US PG-Pub 2007/0065461) who states that the glycoprotein compounds shown in Szymanski et al (1999) (see Figure 7, page 1027, frames A and B, lane 1, 28 –30 kDa) comprise a heptassacharide:"[Guerry et al, 0008] One of the most unusual aspects of C. jejuni is the presence of a general system for N-linked glycosylation of numerous proteins (Szymanski et al., 1999; reviewed in Szymanski et al., 2003). This system, which includes an oligosaccharide transferase similar to that found in the eukaryote Saccharomyces cerevisiae, attaches a glycan which has recently been shown to be **a heptasaccharide** composed of one bacillosamine residue (an unusual deoxy sugar), one D-glucose, and five D-GalNAc residues (Young et al., 2002). The glycosylation appears to occur on numerous periplasmic, and perhaps, surface exposed proteins in C. jejuni (Young et al., 2002). The unusual glycan, again, appears to be highly immunogenic and is recognized during human infection **(Szymanski et al., 1999,** 2003)."

Instant claim 2: glycan compounds are lined to an amino acid (see Szymanaki et al, page 1027, Discussion, col. 1 line 3 "glycosylation of numberous proteins"), wherein the "glycosyl moieties

may be immunodominant (Szymanaki et al, page 1027, col. 2, last sentence bridging to page 1028):

Instant claim 4-5: compounds from Campylobacter jejuni that are immunologically active (see Szymanaki et al , figure 7, page 1027 "Immunoblot)

10. While Szymanaki et al recognized the presence of glycosylation on Campylobacter jejuni proteins, and isolated and purified the glycoproteins by electrophoresis, Szymanaki et al did not describe the characteristics of the glycan groups present on the proteins, but

in light of evidence provided by Guerry et al (US PG-Pub 2007/0065461) who states that the glycoproteins shown in Szymanski et al (1999) (see Figure 7, page 1027, frames A and B, lane 1, 28 – 30 kDa) comprise a heptassacharide: [Guerry et al, 0008] , the **heptasaccharide** composing one bacillosamine residue (an unusual deoxy sugar), one D-glucose, and five D-GalNAc residues, the disclosure of Szymanaki et al anticipates the instantly claimed invention because Szymanaki et al isolated the compound that comprised the unusual glycan, the glycan being highly immunogenic and is recognized during human infection (**Szymanski et al., 1999,** 2003)." The compound of Szymanski et al inherently anticipates the instantly claimed invention as now claimed.

Atlas Powder Co. V IRECA, 51 USPQ2d 1943, (FED Cir. 1999) states Artisans of ordinary skill may not recognize the inherent characteristics or functioning of the prior art...However, *the discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior arts functioning, does not render the old composition patentably new* to the discoverer. The Court further held that this same reasoning holds true when it is not a property but an ingredient which is inherently contained in the prior art.

11. Claims 1-2, 7 and 9 are rejected under 35 U.S.C. 102(e) as being anticipated by Bay et al (filing date 1999)

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

12. Bay et al disclose a compound that comprises a fragment of the compound of claim 1, specifically alpha-GalNAc linked to an amino acid, the amino acid being either threonine or Serine (see claims 1-3), the conjugate being formulated into an immunogenic compositions, with a suitable carrier and adjuvant (see claims 2-3). Bay et al anticipates the instantly claimed invention directed to compounds that comprise fragments of the compound of claim 1.

13. Claims 1-3 are rejected under 35 U.S.C. 102(e) as being anticipated by Pugia et al (filed June 12, 2002).

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

Pugia et al discloses the instantly claimed invention directed to a compound that comprises an immunologically active fragment, the fragment being GalNAc linked to asparagines (Asn) in an oligopeptide (see page 2, [0021] Y= GalNAc and X=Amino acid the compound being GalNAc-Asn-AminoAcid-Ser). Pugia et al anticipates the instantly claimed compound as now claimed.

14. Claims 1-2, 7 and 9 are rejected under 35 U.S.C. 102(b) as being anticipated by Nilsson et al (Publication date 2000).

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

15. Nilsson et al disclose a compound that comprises a fragment of the compound of claim 1, specifically alpha-GalNAc linked to an amino acid, the amino acid being either threonine or Serine (see page 3, bottom half of page, lines 42-55, [0010]), the conjugate being formulated into a composition [0026], with a suitable carrier (see 5, line 17 "water with buffer salts") and is disclosed to further comprise an additional peptide, protein or other spacer molecule [0019] . Nilsson et al anticipates the instantly claimed invention directed to compounds that comprise fragments of the compound of claim 1.

16. Claims 1-2 rejected under 35 U.S.C. 102(b) as being anticipated by Messner et al (1990)

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

Messner et al (1990) disclose a compound that comprises GalNAc-a1,3-Bac (see abstract, BacNAc and page 2580, col. 2, middle of paragraph), wherein the compound comprising GalNAc-a1,3-Bac is linked to an amino acid in peptide (see Table 1, "Ser" and "Asx").

Messner et al anticipates the instantly claimed invention directed to compounds that comprise fragments of the compound of claim 1, the fragment comprising GalNAc-a1,3-Bac .

17. Claims 1-2 rejected under 35 U.S.C. 102(b) as being anticipated by US Pat. 5,840,547 in light of evidence provided by Gutnick et al (US Pat. 6,512,014).

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

US Pat. 5,840,547 is close a Bac compound referred to a emulsan,(in light of evidence provided by Gutnick et al (Brief summary test, paragraph 6)emulsan is a compound that comprises Bac, also known as bacillosamine), wherein US Pat. 5,840,547 discloses Bac (emulsan) covalently linked to an amino acid, specifically alanine (see Brief summary text, paragraphs 7 and 12 of US Pat. 5,840,547).

US Pat. 5,840,547 anticipates the instantly claimed invention directed to compounds that comprise fragments of the compound (Bac) of claim 1 linked to an amino acid (alanine).

18. Claims 1, and 7-9 are rejected under 35 U.S.C. 102(b) as being anticipated by Kaplan et al (WO 00/51635) in light of evidence provided by Gutnick et al (US Pat. 6,512,014).

Please Note: the following art rejection is being applied to the claims based upon the recitation of ---immunologically active fragment thereof-----.

(Instant claim 1)Kaplan et al disclose a compound that comprises Bac, specifically emulsan (see '635, page 2, line 11) (Gutnick et al provide evidence (Brief summary test, paragraph 6) that emulsan is a compound that comprises Bac, also known as bacillosamine), formulated together with an antigen (see page 6, lines 10-11)

Instant claim 7: into a pharmaceutical composition, the composition further comprising a physiologically acceptable carrier (see page 18, lines 28-30 " in combination with other physiologically acceptable medium (e.g., water, buffered saline, polyols such as glycerol, propylene glycol, liquid polyethylene glycol and dextrose solutions)",

Instant claim 8: and immunoconjugate (see '635, page 8, lines 10-13 : antigen linked to an additional carrier such as bovine serum albumin (BSA) or keyhole limpet hemoxyanin (KLH); and claim 13, page 41)

Instant claim 9: the antigen carrier being an immunostimulant (KLH).

Kaplan et al anticipates the instantly claimed invention as now claimed.

Conclusion

19. This is a non-final action.

20. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Various references are being made of record to show bacterial glycoproteins.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ginny Portner whose telephone number is (571) 272-0862. The examiner can normally be reached on flextime, but usually M-F, alternate Fridays off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on (571) 272-0787. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Vgp
August 1, 2007

MARK NAVARRO
PRIMARY EXAMINER

Notice to Comply	Application No. 10/523459	Applicant(s) Young et al
	Examiner Portner	Art Unit 1645

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant must file the items indicated below within the time period set in the Office action to which the Notice is attached to avoid abandonment under 35 U.S.C. § 133 (extensions of time may be obtained under the provisions of 37 CFR 1.136(a)).

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

☒ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).

☒ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).

☒ 7. Other: Additional Sequences have been found; find narrative in attached document.

Applicant Must Provide:

☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".

☒ An initial or substitute paper copy of the "Sequence Listing", **as well as an amendment specifically directing its entry into the application**.

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (571) 272-2510
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times from 30-151 ms and 1D NOESY with mixing times from 400-800 ms were performed as described previously (25,26). Selective experiments were described as 1D EXP(selected spins, selective excitation bandwidth, mixing time) where EXP is TOCSY or NOESY.

The use of magic angle spinning (MAS) for liquid state samples in the presence of both RF and magnetic-field homogeneities has been shown to influence significantly the performance of mixing sequences in TOCSY experiments and can degrade performance (27,28). Using adiabatic (WURST) mixing sequences can eliminate such effects (27,29). The standard 2D TOCSY and 1D TOCSY sequences were modified so that the MLEV-17 or DIPSI-2 mixing sequence was replaced with the adiabatic WURST-2 pulses. The adiabatic (WURST-2) mixing had a single adiabatic inversion pulse length of T_p= 1/MAS spin rate, a modulation depth of 8 and an adiabicity of 2. Typically, for the WURST-2 pulse, the sweep bandwidth was 24 kHz, T_p = 0.333 ms (at a MAS spin rate of 3000 +/- 10 Hz), B_1 (max)= 8.51 kHz, B_1 (RMS) = 4.77 kHz.

GC-MS analysis: The enantiomeric configurations of the Glc and GalNAc components of the P2 product were assigned by characterization of the but-2-yl glycosides in gas liquid chromatography – mass spectrometry (21). The derivatives were analyzed using a Hewlett-Packard chromatograph equipped with a 30m DB-17 capillary column (180°C to 260°C at 3.5°C/min), and spectra in the electron impact mode were obtained with a Varian Saturn II mass spectrometer.

Construction and characterization of *pglB* mutant: For construction of the *pglB* mutant, genes Cj1121c to Cj1126c were PCR amplified from *C. jejuni* NCTC 11168 using the primers: Cj1121cF (5'-ACTCACTATTGCCATTAAGATAAGC-3') and Cj1126cR (5'-AAAACCCTTATTTAGTTTTGTTTGC-3'). The PCR product was polished with *Pfu* polymerase and then ligated into pPCR-Script Amp (Stratagene) according to the manufacturer's instructions. The ligation mixture was electroporated into electrocompetent *E. coli* DH10B and selected for on LB S-gal agar (Sigma-Aldrich) with ampicillin. A blunt-ended kanamycin resistance cassette from pILL600 (37) was inserted into the filled-in *Xba*I restriction site of *pglB*, generating pEAp26. The orientation of the cassette was determined by

sequencing with the ckanB primer (5'-CCTGGGTTTCAAGCATTAG-3'). DNA was sequenced using terminator chemistry and AmpliTaq cycle sequencing kits (Applied Biosystems) and analysed on an Applied Biosystems 373 DNA sequencer. The mutated plasmid DNA was used for electroporation into *C. jejuni* NCTC 11168 (32) and the kanamycin-resistant transformants were characterized by PCR to confirm that the incoming plasmid DNA had integrated by a double cross-over event.

Proteins were extracted from *C. jejuni* whole cells using 0.2 M glycine at pH 2.2 (10) and dialysed against water. Samples were analyzed by 2D-PAGE using 11 cm pH 3-10 ReadyStrip IPG strips (BioRad Laboratories) and pre-cast 12 x 8 cm 8-16% gradient Criterion slab gels (BioRad Laboratories). Gels were stained with colloidal coomassie blue, photographed, and then partially destained by washing in water. Proteins were transferred for 1 h at 207 mA onto PVDF membranes using a Trans-Blot SD Semi-Dry Transfer Cell (BioRad). After blocking overnight, membranes were probed with a 1:500 dilution of HS:2 serotyping serum followed by a 1:5000 dilution of goat anti-rabbit antiserum (Sigma-Aldrich), and developed with NBT/BCIP (Roche Molecular Biochemicals).

EXPERIMENTAL PROCEDURES FOR THE DETECTION OF GLYCANS FROM CAMPYLOBACTER CELLS

Bacterial strains and growth conditions: *Campylobacter jejuni* NCTC11168 (HS:2) was isolated from a case of human enteritis (46) and later sequenced by Parkhill *et al.* (6). *C. jejuni* serostrains: HS:1 (ATCC 43429), HS:2 (ATCC 43430), HS:3 (ATCC 43431), HS:4 (ATCC 43432), HS:10 (ATCC 43438), HS:19 (ATCC 43446), HS:36 (ATCC 43456) and HS:41 (ATCC 43460) were obtained from ATCC; *C. jejuni* HS:23 was obtained from Dr. Peggy Godschalk, Erasmus University Medical Center, Rotterdam; *C. jejuni* OH4382 and OH4384 were obtained from Health Canada; and *C. coli* HS:30 (NCTC 12532) was obtained from NCTC. All campylobacter strains were routinely

is TOCSY or NOESY. Typically, proton spectra of bacterial cells could be obtained using 256 to 1024 transients (15 min to 1 hour). For the selective experiments on the *N*-linked glycan resonances present as a minor component in the bacterial cells, the time for each TOCSY and NOESY varied from 1 to 8 hours.

EXAMPLES

EXAMPLE 1 - Purification and characterization of PEB3

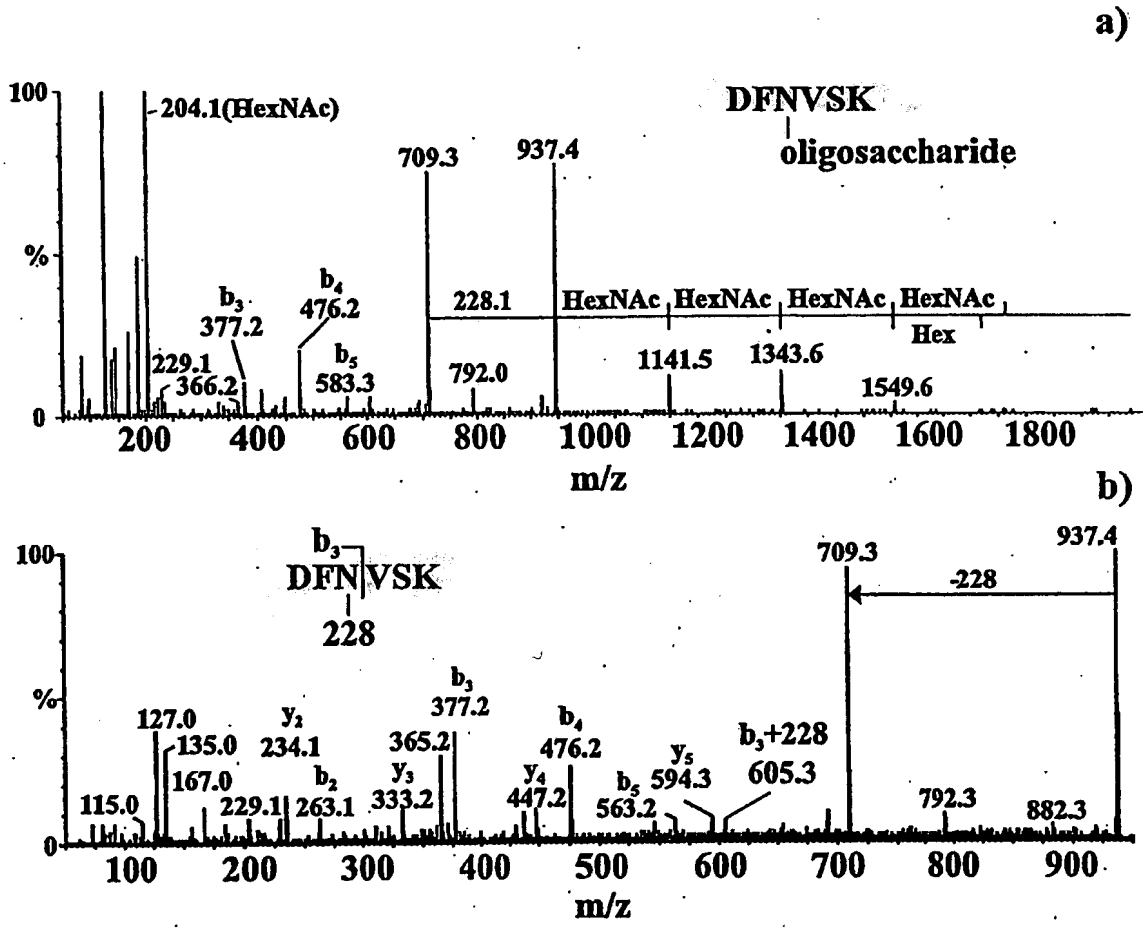
PEB3 protein (Cj0289c) was identified in 2D gels of a glycine extract by peptide mass fingerprinting, as a component of a group of spots focussing within a range of pH 9-10 (results not shown). PEB3 was purified from the extract by cation exchange chromatography, and re-fractionated on the same column, using a shallower NaCl gradient, resulted in the PEB3 appearing in three fractions (Fig.1). SDS-PAGE analysis showed two bands, whose *N*-terminal sequences were determined following their transfer to a PVDF membrane. Ten cycles of sequencing identified the lower mass species as PEB3 while the higher mass, more abundant component, was also PEB3 with a minor sequence corresponding to PEB4 (Cj0596).

The mass spectrum and the reconstructed molecular mass profile for fraction # 31 are presented in Figure 2 a) and b). Three peaks were observed in the reconstructed mass profile. The peaks at 25,454 Da and 28,376 Da correspond well with the expected molecular masses of PEB3 (25,453 Da, Cj0289c) and PEB4 (28,377 Da, Cj0596) respectively, without signal peptides. To identify the protein of mass 26,861 Da, CapLC-MS/MS analysis was carried out on the tryptic digest of this fraction. All but one of the peptides identified could be assigned to PEB3 or PEB4, in accord with the *N*-terminal sequence data. MS/MS analysis of the unidentified ion (Fig. 3a) clearly identifies it as a glycopeptide. A fragmentation series composed of sequential losses of HexNAc (203 Da) and a single Hex (162 Da) can be observed in this spectrum. The tryptic peptide was identified as ^{68}DFNVSK^{73} from PEB3. The

that the proteins purified in this manner possessed lectin binding properties, rather than non-specific binding characteristics, western blotting with an SBA/alkaline phosphatase conjugate was also carried out. Approximately 13 protein species were visualized following 1D SDS-PAGE but this number increased substantially when the product was analyzed by 2D-PAGE. The proteins in individual bands from 1D SDS-PAGE and spots from 2D-PAGE were identified by mass fingerprinting and database searching (Table I). Among the identified proteins are PEB3 (Cj0829c) and CgpA (Cj1670c) previously identified by Linton *et al.* (8). The vertical pattern of spots with identical pIs displayed by Cj1670c, and other proteins, likely indicates varying degrees of glycosylation since examination of their predicted amino acid sequences, derived from the whole genome sequence of *C. jejuni* NCTC 11168 (6), revealed the presence of multiple potential *N*-linked glycosylation sites containing the sequon Asn-Xaa-Ser/Thr (Table I). In fact, MS/MS analysis of the Cj1670c-containing in-gel digest extracts indicated that 3 of its 6 *N*-linkage sites are occupied to varying extents (three Cj1670c glycopeptides were detected by capLC-MS/MS: ^{7}TDQNITLVAPPEFQKEEVK^{25}, ^{77}VLDVSVTIPEKNSSK^{91} and ^{92}QESNSTANVEIPLQVAK^{108}. A single glycopeptide was also observed for Cj0114 (^{71}LSQVEENNQNIENNFTSEIQK^{91}) and for Cj0200c (^{1}DSLKLEGTIAQIYDNNK^{17}). Furthermore, the mass and composition of the glycan component of all these glycopeptides appears to be identical to that observed for PEB3.

However, certain proteins identified from the 2D-PAGE, notably Cj0147c, Cj0169, Cj0332c, Cj0334, Cj0638c, Cj1181c and Cj1534c do not contain any of these specific sequons in their amino acid sequences. These proteins either are non-covalently associated with SBA-binding proteins or bind non-specifically to the column. This conclusion is supported by the failure of these protein spots to react with the SBA/alkaline phosphatase conjugate following 2D PAGE and electroblotting (Table I).

Figure 3


a)
100
%
0
204.1(HexNAc)
DFNVSK
oligosaccharide
709.3
937.4
b3
377.2
b4
476.2
228.1
HexNAc
HexNAc
HexNAc
HexNAc
Hex
229.1
366.2
b5
583.3
792.0
1141.5
1343.6
1549.6
200
400
600
800
1000
1200
1400
1600
1800
m/z
b)
100
%
0
b3
DFN|VSK
228
709.3
937.4
-228
b3
377.2
127.0
135.0
167.0
115.0
y2
234.1
b2
229.1
263.1
365.2
y3
333.2
b4
476.2
y4
447.2
b5
563.2
y5
594.3
b3+228
605.3
792.3
882.3
100
200
300
400
500
600
700
800
900
m/z